

DIAGENESIS AND SURVIVAL OF INTRACRYSTALLINE AMINO ACIDS IN FOSSIL AND RECENT MOLLUSC SHELLS

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ABSTRACT. Amino acid analysis was carried out on intracrystalline organic material from fossil and recent mollusc shells from South Wanganui Basin, New Zealand, ranging in age from 3.6 My old to Recent. The absolute abundance of amino acids is highly variable but shows a gradual decline through time due to diagenetic effects. The proportion of peptide-bound amino acids decreases with time, and there is a corresponding increase in free amino acids as proteins are broken down by natural hydrolysis. By 0.5 Ma, most amino acids are free, and the rate of decay of peptide bonds appears to slow appreciably, with small proportions of peptide bound amino acids occurring in shells throughout the time span investigated. The quantities of free amino acids reach a peak between 0.5 and 1 Ma, after which there is a general decrease in most individual amino acids, presumably because they decay or become incorporated into predominantly insoluble geopolymers. Alanine is a notable exception, increasing in older samples because it is a common by-product of the breakdown of other amino acids.

Amino acid data from different species and from shell beds of different ages were compared using multivariate statistical techniques. The results indicate that, despite the effects of diagenesis, the original biochemical distinction between different groups of molluscs (i.e. different proteins within the shell) survives for at least 3.6 My, and may be detectable in older specimens provided sufficient original amino acids remain.

IN order to gain meaningful information from organic molecules in fossil shells, it is necessary to isolate original organic matter which has not been contaminated or replaced by more recent biomolecules. For this purpose, intracrystalline biomolecules are used. These molecules are trapped within the crystals of the shell during biomineralization and are therefore protected from contamination by outside sources until released by decalcification. Many of these molecules may have been actively involved in the process of shell formation by acting as a nucleation site for crystal growth (Lowenstam and Weiner 1989). The molecules which are involved in the biomineralization process are usually proteins and protein-like macromolecules secreted by the cells of the mantle, which form a matrix within and around which calcium carbonate crystals are precipitated (Lowenstam and Weiner 1989). Each protein molecule consists of a chain of amino acids, the order of which is determined by the sequence of nucleotides in the DNA which controls its production. These amino acids are therefore directly related to genotype and may represent useful taxonomic indicators even after the protein sequence has been destroyed (Walton 1992). The interpretation of amino acid data from fossil shells, however, is complicated by the fact that individual amino acids vary in stability, and can decompose at varying rates into other compounds or into other amino acids (Vallentyne 1964, 1968). Hence the amino acid compositions of fossil shells are likely to be less easy to interpret and will reflect complicated amino acid diagenesis reactions as well as original taxonomic variability. The purpose of this paper is to analyse both the peptide bound and the free (i.e. naturally hydrolysed) amino acids from within shells of different ages, in order to investigate the state of preservation of intracrystalline proteins and the extent to which original biochemical differences between taxonomic groups survives the fossilization process.

TEXT-FIG. 1. Map showing locations of collection sites.



LOCALITIES AND METHODS

Fossil mollusc shells were collected from sea cliffs and inland exposures in the South Wanganui Basin, New Zealand (Text-fig. 1). This area was chosen for its almost continuous sequence of late Pliocene and Pleistocene fossiliferous shallow marine deposits containing abundant mollusc shells. The sequence consists of poorly consolidated layers of sand and mud deposited mainly during interglacials and separated by unconformities associated with glacial marine regressions. Recent mollusc shells were collected from near shore locations and from beaches around the coasts of the North and South Islands of New Zealand (Text-fig. 1).

Well preserved shells of various species were selected from shell beds at different levels in the Wanganui sequence (Text-fig. 2) along with related Recent species. Several specimens were taken from each of 28 shell beds, making 60 specimens in all (Table 1). Each specimen was examined by SEM to check for signs of recrystallization, and XRD analysis was used to check that aragonite shells had not reverted to calcite, as such recrystallization would have allowed contamination of intracrystalline biomolecules. The shells were cleaned of sediment and algae and the ligaments removed. Where necessary the shells were placed in a cool oven at 40 °C to dry out the periostracum which was then removed. Each shell was washed in water then soaked in an aqueous solution of bleach to remove any remaining organic molecules from the surface by oxidation. The shells were then rinsed in clean Milli Ro™ water and left to air dry.

Small carbonate samples were taken from each shell using a small drill with a rotating tip with a diameter of 1 mm. Intercrystalline organic matter was removed from the carbonate powder by

Stage	Substage	Formation	Sample No.
Haweran		Alluvium	17,18
		Rapanui Formation Brunswick Formation Kaiatea Formation Landguard Formation	5,19,20,21
Castlecliffian	Putikian	Putiki Shellbed Mosstown Sand Karaka Siltstone Upper Castlecliff Shellbed Shakespeare Cliff Sand Shakespeare Cliff Siltstone Tainui Shellbed Pinnacle Sand Lower Castlecliff Shellbed Seafield Sand Upper Kai Iwi Siltstone Kupe Formation	22,23,24 25,26,27,28,29 30,31,32 33,34,35 36,37,38 39 8,40,41,42
	Okehuan	Upper Westmere Siltstone Kaikokapu Formation Lower Westmere Siltstone Ophiomorpha Sand Omapu Shellbed Lower Kai Iwi Siltstone Kaimatira Pumice Sand Upper Okehu Siltstone Okehu Shell Grit Lower Okehu Siltstone Mowhanau Formation Ototoka Siltstone Butler's Shell Conglomerate	47,48 43,44 45,46 49 50 51,52,53 54,55
Nukumaruan	Marahauan	Upper Maxwell Formation Mangahou Siltstone Middle Maxwell Formation Pukekiwi Shell Sand Lower Maxwell Formation Tewkesbury Formation Waipuru Shellbed Nukumaru Brown Sand Mangamako Shellbed Nukumaru Limestone Ohingaiti Sand	56 57 58,59,60 14
	Hautawan	Undifferentiated Formations Kuranui Limestone Hautawa Shellbed	61
Waitotaran	Mangapanian	Te Rama Shellbed Parihauhau Shellbed Te Rimu Sand Wilkies Shellbed Makokako Sand Mangaweka Mudstone Paparangi Sandstone	62
	Waipipian	Waverley Formation Upper Waipipi Shellbed Middle Waipipi Shellbed Lower Waipipi Shellbed Snapper Point Shellbed Rangikura Sandstone Pepper Shell Sand	13,65,66 63,67 68 4 1,2,3

TEXT-FIG. 2. Stratigraphical column of the Wanganui Series, after Fleming (1953) and Abbott and Carter (1991), showing sample points.

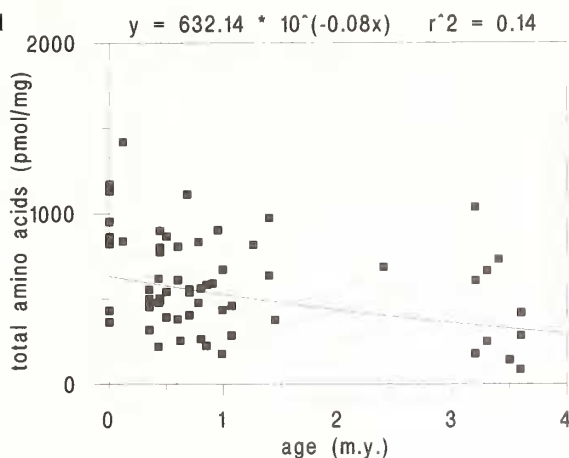
TABLE 1. Shell samples 1–60: species, superfamilies, locations and ages.

1.	<i>Maoricardium spatiosum</i> (Cardiacea) – Pepper Shell Sand (c. 3.6 Ma)
2.	<i>Phialopecten marwicki</i> (Pectinacea) – Pepper Shell Sand (c. 3.6 Ma)
3.	<i>Maoricardium spatiosum</i> (Cardiacea) – Pepper Shell Sand (c. 3.6 Ma)
4.	<i>Maoricardium spatiosum</i> (Cardiacea) – Snapper Point Shell bed (c. 3.5 Ma)
5.	<i>Pecten tainui</i> (Pectinacea) – Landguard Formation (c. 0.35 Ma)
6.	<i>Anchomasa similis</i> (Pholadacea) – Castlecliff Beach (Recent)
7.	<i>Paphies (Mesodesma) subtriangulata</i> (Mesodesmatacea) – Long Beach (recent)
8.	<i>Tiostrea chilensis</i> (Ostreacea) – Kupe Shell bed (c. 0.7 Ma)
9.	<i>Paphies australis</i> (Mesodesmatacea) – Te Rauone Beach (Recent)
10.	<i>Chione (Austrovenus) stutchburyi</i> (Veneracea) – Warrington Beach (Recent)
11.	<i>Maoricolpus roseus</i> (Cerithiacea) – Long Beach (Recent)
12.	<i>Maoricardium spatiosum</i> (Cardiacea) – Upper Waipipi (c. 3.2 Ma)
13.	<i>Patro undatus</i> (Anomiacea) – Nukumarū Limestone (c. 1.45 Ma)
14.	<i>Chione (Austrovenus) stutchburyi</i> (Veneracea) – Te Rauone Beach (Recent)
15.	<i>Maetra discors</i> (Mactracea) – Warrington Beach (Recent)
16.	<i>Spisula (Crassula) aequilateralis</i> (Mactracea) – Rapanui Formation (c. 0.12 Ma)
17.	<i>Paphies (Mesodesma) subtriangulata</i> (Mesodesmatacea) – Rapanui Formation (c. 0.12 Ma)
18.	<i>Tawera spissa</i> (Veneracea) – Landguard Formation (c. 0.35 Ma)
19.	<i>Tiostrea chilensis lutaria</i> (Ostreacea) – Landguard Formation (c. 0.35 Ma)
20.	<i>Pecten tainui</i> (Pectinacea) – Landguard Formation (c. 0.35 Ma)
21.	<i>Zethalia zelandica</i> (Trochacea) – Upper Castlecliff Shell bed (c. 0.43 Ma)
22.	<i>Paphies (Mesodesma) subtriangulata</i> (Mesodesmatacea) – Upper Castlecliff Shell bed (c. 0.43 Ma)
23.	<i>Venericardia purpurata</i> (Carditacea) – Upper Castlecliff Shell bed (c. 0.43 Ma)
24.	<i>Paphies (Mesodesma) subtriangulata</i> (Mesodesmatacea) – Shakespeare Cliff Sand (c. 0.44 Ma)
25.	<i>Pecten tainui</i> (Pectinacea) – Shakespeare Cliff Sand (c. 0.44 Ma)
26.	<i>Tiostrea chilensis lutaria</i> (Ostreacea) – Shakespeare Cliff Sand (c. 0.44 Ma)
27.	<i>Paphies (Mesodesma) subtriangulata</i> (Mesodesmatacea) – Shakespeare Cliff Sand (c. 0.44 Ma)
28.	<i>Venericardia purpurata</i> (Carditacea) – Shakespeare Cliff Sand (c. 0.44 Ma)
29.	<i>Tiostrea chilensis lutaria</i> (Ostreacea) – Tainui Shell bed (c. 0.5 Ma)
30.	<i>Maoricolpus roseus</i> (Cerithiacea) – Tainui Shell bed (c. 0.5 Ma)
31.	<i>Venericardia purpurata</i> (Carditacea) – Tainui Shell bed (c. 0.5 Ma)
32.	<i>Pecten tainui</i> (Pectinacea) – Lower Castlecliff Shell bed (c. 0.6 Ma)
33.	<i>Maoricolpus roseus</i> (Cerithiacea) – Lower Castlecliff Shell bed (c. 0.6 Ma)
34.	<i>Venericardia purpurata</i> (Carditacea) – Lower Castlecliff Shell bed (c. 0.6 Ma)
35.	<i>Venericardia purpurata</i> (Carditacea) – Tom's Conglomerate (c. 0.62 Ma)
36.	<i>Tiostrea chilensis lutaria</i> (Ostreacea) – base of Upper Kai-Iwi Siltstone (c. 0.68 Ma)
37.	<i>Maoricolpus roseus</i> (Cerithiacea) – Upepe Formation (c. 0.7 Ma)
38.	<i>Venericardia purpurata</i> (Carditacea) – Kupe Formation (c. 0.7 Ma)
39.	<i>Paphies (Mesodesma) subtriangulata</i> (Mesodesmatacea) – Kaikōkapu Formation (c. 0.78 Ma)
40.	<i>Venericardia purpurata</i> (Carditacea) – Kaikōkapu Formation (c. 0.78 Ma)
41.	<i>Divaricella (Divalucina) huttoniana</i> (Lucinacea) – Omapu Shell bed (c. 0.85 Ma)
42.	<i>Amalda (Baryspira) mucronata</i> (Muricacea) – Omapu Shell bed (c. 0.85 Ma)
43.	<i>Amalda (Baryspira) mucronata</i> (Muricacea) – Upper Westmere Shell bed (c. 0.8 Ma)
44.	<i>Maoricolpus roseus</i> (Cerithiacea) – Upper Westmere Shell bed (c. 0.8 Ma)
45.	<i>Paphies (Mesodesma) subtriangulata</i> (Mesodesmatacea) – Lower Kai-Iwi Shell bed (c. 0.9 Ma)
46.	<i>Paphies (Mesodesma) subtriangulata</i> (Mesodesmatacea) – Kaimatira Pumice Sand (c. 0.95 Ma)
47.	<i>Maoricrypta (Zeacrypta) monoxyla</i> (Calyptreaeacea) – Okehu Shell Grit (c. 0.99 Ma)
48.	<i>Tiostrea chilensis lutaria</i> (Ostreacea) – Okehu Shell Grit (c. 0.99 Ma)
49.	<i>Venericardia purpurata</i> (Carditacea) – Okehu Shell Grit (c. 0.99 Ma)
50.	<i>Maoricrypta (Zeacrypta) monoxyla</i> (Calyptreaeacea) – Butler's Shell Conglomerate (c. 1.07 Ma)
51.	<i>Chlamys gemmulata</i> (Pectinacea) – Butler's Shell Conglomerate (c. 1.07 Ma)
52.	<i>Austrovenus stutchburyi</i> (Veneracea) – Mangahou (c. 1.26 Ma)
53.	<i>Patro undatus</i> (Anomiacea) – top of Nukumarū Brown Sand (c. 1.4 Ma)
54.	<i>Lutraria solida</i> (Mactracea) – Nukumarū Brown Sand (c. 1.4 Ma)

TABLE 1. (cont.)

55.	<i>Patro undatus</i> (Anomiacea) – Hautawa shell bed (c. 2.4 Ma)
56.	<i>Crassostrea ingens</i> (Ostreacea) – Middle Waipipi (c. 3.3 Ma)
57.	<i>Lima waipipiensis</i> (Limacea) – Upper Waipipi (c. 3.2 Ma)
58.	<i>Crassostrea ingens</i> (Ostreacea) – Upper Waipipi (c. 3.2 Ma)
59.	<i>Maoricardium spatiosum</i> (Cardiacea) – Middle Waipipi (c. 3.3 Ma)
60.	<i>Crassostrea ingens</i> (Ostreacea) – Lower Waipipi (c. 3.4 Ma)

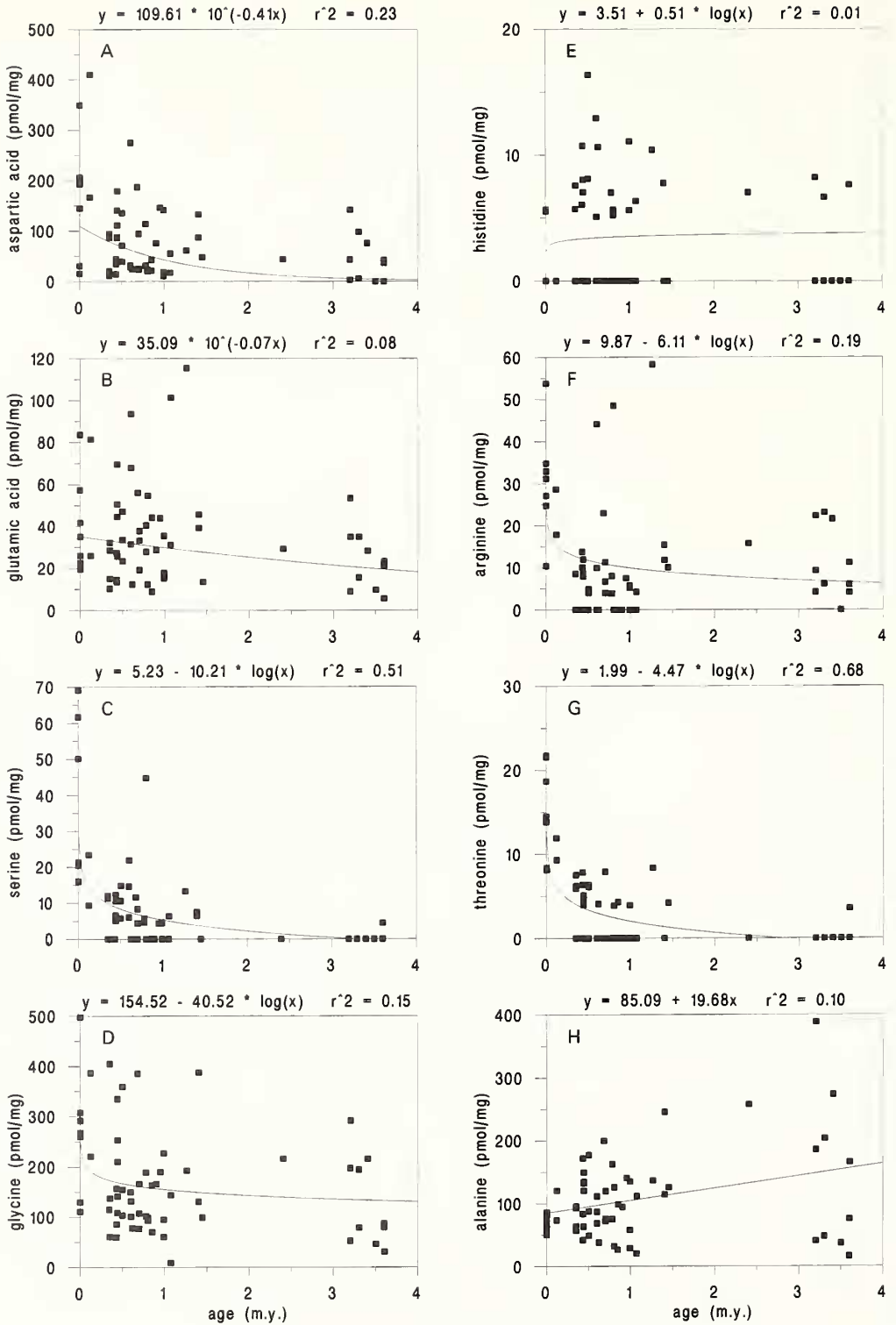
TEXT-FIG. 3. Total intracrystalline amino acids in all samples plotted against sample age.



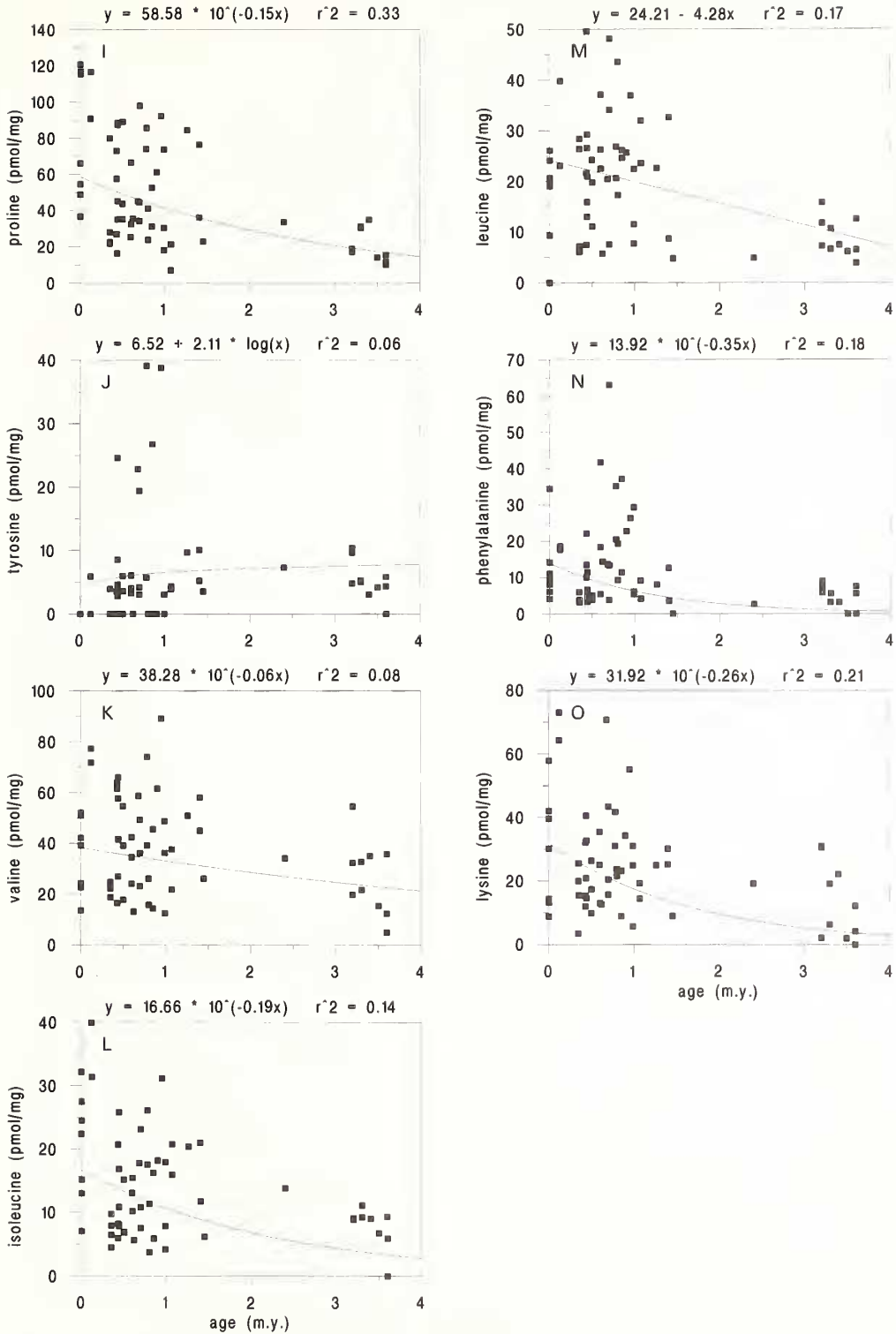
plasma ashing. The samples were dissolved in 2N hydrochloric acid at a ratio of 11 μ l per mg of shell and centrifuged to remove insoluble compounds. Analysis was carried out using an Applied Biosystems 420H amino acid analyser. Each sample was analysed both with and without hydrolysis in order to determine the proportion of amino acids present in the free state, i.e. the extent to which the intracrystalline proteins in each shell had been naturally hydrolysed.

Some common proteinogenic amino acids are easily destroyed by hydrolysis. These include asparagine and glutamine (these get hydrolysed to aspartic acid and glutamic acid), methionine and cysteine (these break down due to the oxidation of side-chain sulphur atoms) and tryptophan (this is lost due to the breaking of the carbon double bond present within its ring structure). These reactions are likely to have occurred by natural hydrolysis long before analysis. Therefore, in this study, amounts of original asparagine and glutamine are included in the figures for aspartic acid and glutamic acid respectively, and methionine, cysteine and tryptophan are not quantified. The resulting changes in bulk amino acid composition apply consistently to all samples and therefore do not affect sample relationships. Data are presented as picomoles per milligramme of shell (pmol/mg), which is the form in which the raw data are obtained from the amino acid analyser. This is equivalent to nanomoles per gramme of shell.

The amount of peptide bound amino acids is calculated by subtracting the free amino acids from the total amino acids. Sometimes this results in a negative value, indicating that there are more free amino acids than total amino acids, an impossible conclusion. This problem results from the fact that for total amino acid analysis, peptide bonds must be first hydrolysed using hydrochloric acid. This can result in the total destruction of some amino acids as outlined above. In addition, certain other amino acids may suffer some small losses as a result of this hydrolysis. For example, losses of the amino acids serine, threonine and tyrosine may be of the order of 10–20 per cent. When this problem occurs, and the calculated value for the peptide bound amino acids is negative, the value is taken to be zero.



TEXT-FIG. 4. For caption see opposite.



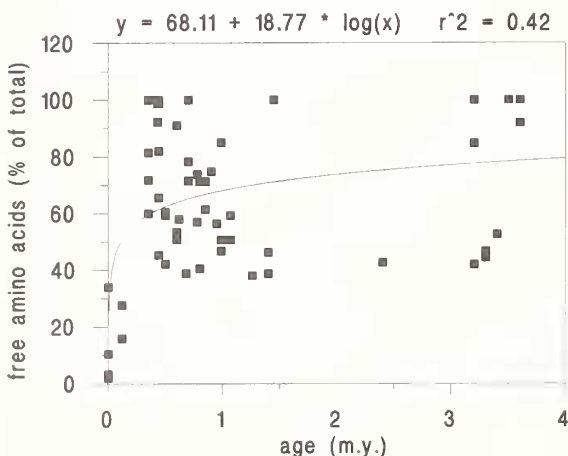
TEXT-FIG. 4. Amount of total individual intracrystalline amino acids plotted against sample age.

The amino acid data were analysed statistically by means of principal component analysis techniques using the program 'Datadesk™' on an Apple Macintosh™ microcomputer. The aim was to investigate the relationships between the amino acid compositions of different samples using a suitable multivariate procedure. In this way a high percentage of the total variance between samples can be expressed on a three dimensional, rotating plot. Groups of samples were chosen that were known to be taxonomically related (e.g. the mollusc superfamilies Cardicea, Carditacea, Pectinacea, Mactracea, Ostreacea, Anomiacea, Mesodesmatacea, Veneracea and Cerithiacea). Groups of samples from three, four or five superfamilies were plotted together according to their amino acid compositions.

CHANGES IN AMINO ACID COMPOSITIONS THROUGH TIME

The total intracrystalline amino acid compositions of all 60 samples are shown in Text-figure 3. The data reveal a high degree of variability, even in recent samples in which the absolute yield of amino acids ranges from about 400 to 1200 pmol/mg. This level of variability is maintained in fossil samples. Statistically there is an overall decline in abundance through time, but the low coefficient of correlation is testimony to the highly variable nature of the amino acid data. Clearly different taxa of molluscs have very different quantities of proteins present in their shells and this is reflected in the variable yields from related fossil taxa. A gradual decline through time is also seen in most individual amino acids with the notable exception of alanine which increases quite significantly (Text-fig. 4). A few amino acids, such as histidine and tyrosine, are present in such small quantities that the pattern is distorted due to the effect of the detection limit of the amino acid analyser (about 5 pmol/mg), which results in a 'gap' in the results below this value.

Free amino acids are rare in Recent samples, but rapidly become an increasingly important component of the total amino acid yield (Text-fig. 5). Many of the individual amino acids show a pattern of rapid initial increase during the first 0.5 My followed by a gradual decline in older specimens, probably due to the decay of amino acids to form compounds that are not detected by the amino acid analyser (Text-fig. 6). Alanine shows a marked increase over the whole period. The total free amino acids show an initial increase followed by a levelling off as the decline in most individual free amino acids is offset by the increase in alanine, this being the most abundant individual free amino acid. The absolute yield of free amino acids from different specimens is, like the total yield, highly variable. As expected, the rapid initial increase in free amino acids is matched by a rapid initial decrease in peptide bound amino acids (Text-fig. 7). This pattern is seen in most individual peptide bound amino acids except histidine and tyrosine, which are present in such small quantities as to show no pattern (Text-fig. 8).



TEXT-FIG. 5. Free intracrystalline amino acids as a percentage of the total intracrystalline amino acids in all samples through time.

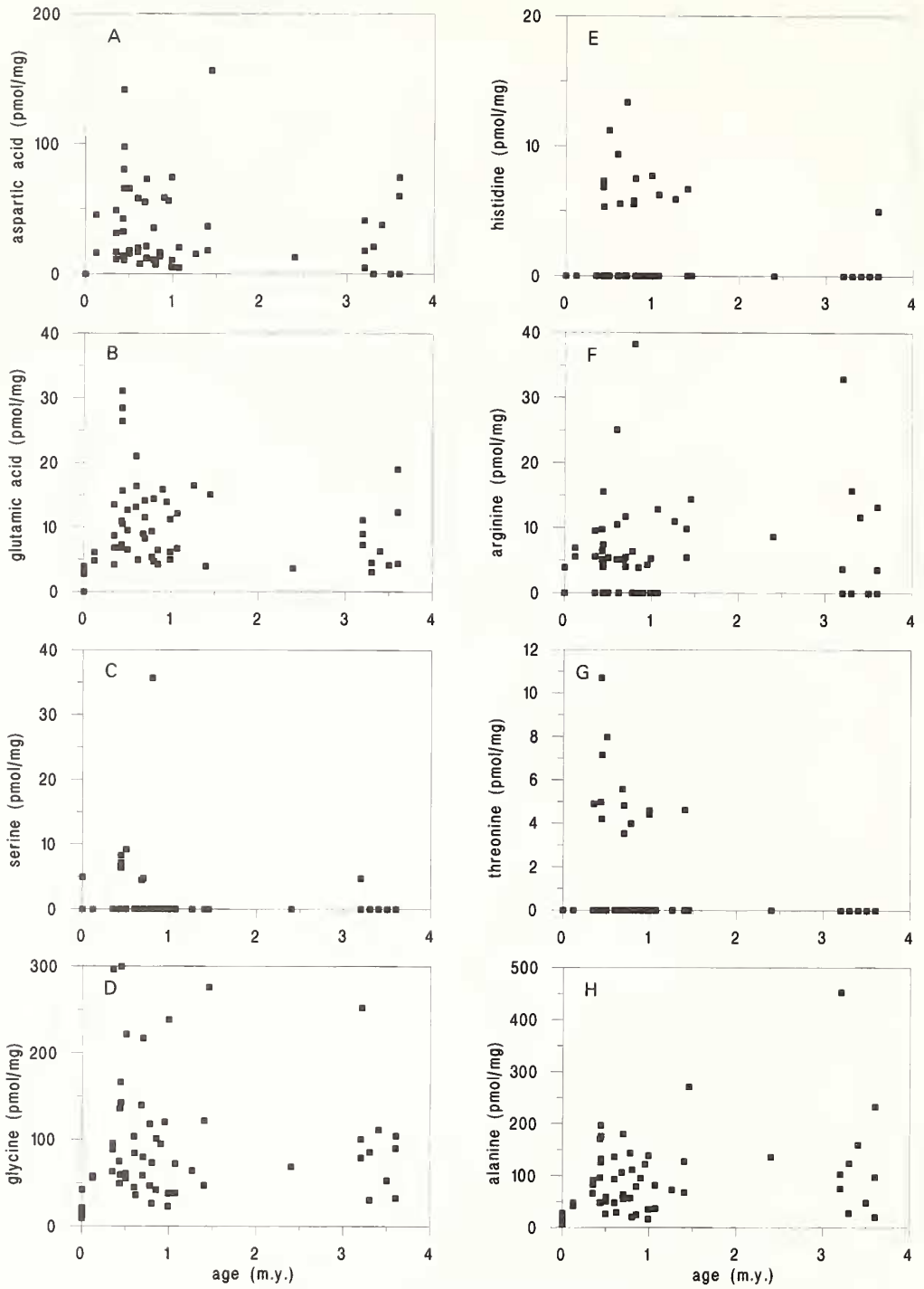
These results are largely as would be expected. The gradual decrease in total amino acids through time is likely to be a function of biochemical reactions which gradually break down some of the amino acids into other compounds which are not detected by the amino acid analyser. In recent shells, the large amounts of peptide bound amino acids and small numbers of free amino acids show that protein preservation is good. The rapid initial increase in free amino acids and corresponding decrease in peptide bound amino acids, however, show that the proteins in fossil shells are poorly preserved as they quickly break down due to natural hydrolysis through time, producing a mixture of free amino acids and other compounds. Most of the peptide bonds are broken within the first 0.5 My. However, some peptide bound amino acids do remain in the oldest samples, indicating that hydrolysis has not been complete and that some peptides may persist for a long time.

These results are consistent with the results of Walton (1992) who found that the proportion of amino acids present in the free state in brachiopod shells rises from negligible amounts in Recent specimens to greater than 58 per cent. by 0.2 Ma, and generally greater than 80 per cent. by 0.5 Ma, showing that brachiopod shell proteins also tend to undergo very rapid initial hydrolysis. Excellent physical preservation therefore, does not necessarily indicate good biochemical preservation (Towe 1980; Weiner and Lowenstam 1980). Previous studies have shown that the rate of this natural hydrolysis is dependent upon many factors: water availability and temperature (and therefore burial history) being amongst the most important (Vallentyne 1964, 1968; Ho 1966). The strength of the peptide bonds, and therefore the rate at which they can be broken, is dependent upon the nature of the residues on either side of the bond (Kleef *et al.* 1975; Kahne and Still 1988; Eglinton and Logan 1991). The rate of hydrolysis is also affected by the presence of other biomolecules, such as carbohydrates, which are present within shells (Vallentyne 1964), and metal ions (Ikawa and Snell 1954), both of which tend to speed up the reactions. The speed of amino acid decomposition reactions, therefore, is very difficult to predict and may not relate directly to the thermal stability of individual pure amino acids as determined by Vallentyne (1964).

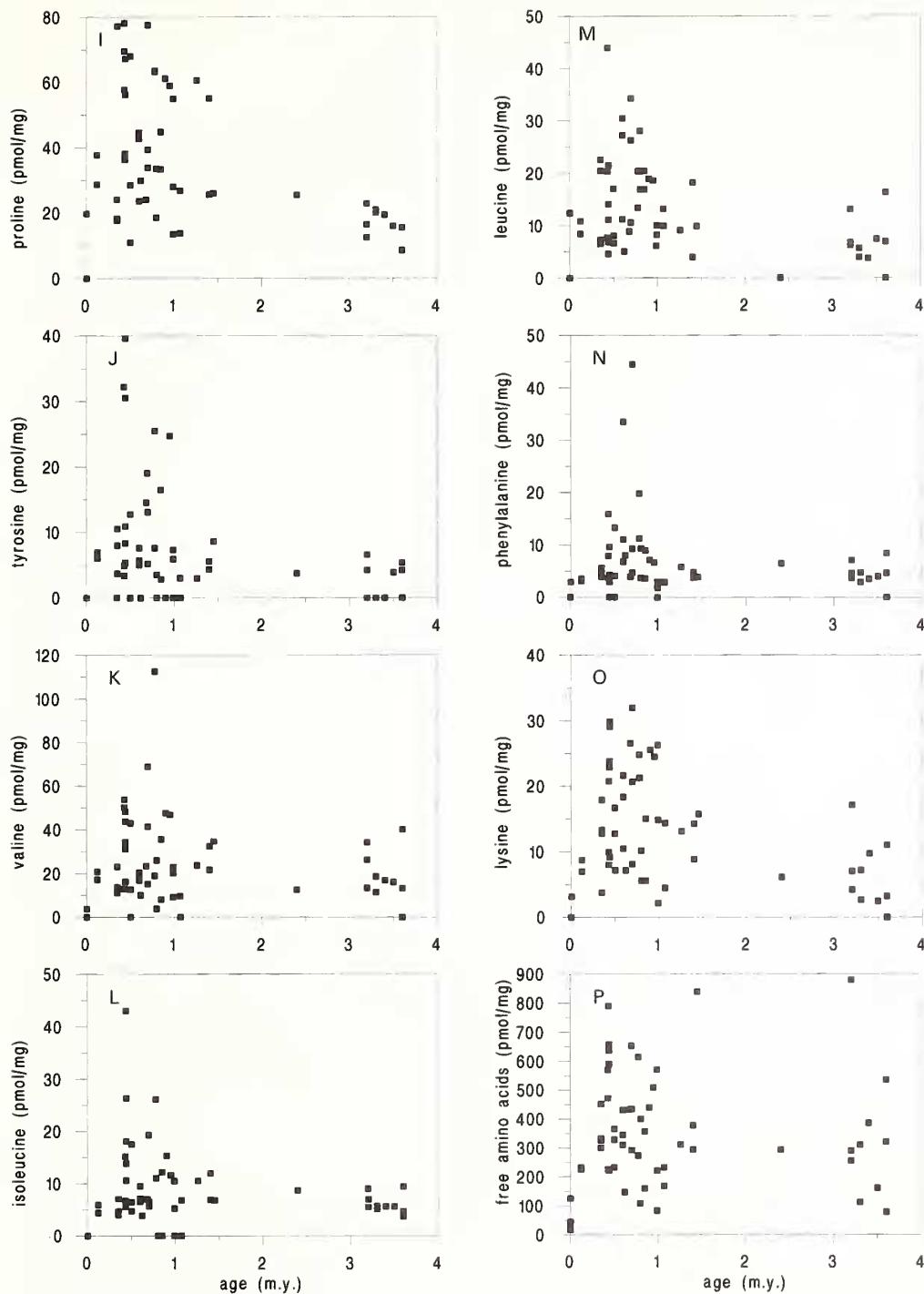
The changes in individual amino acids show that amounts of most amino acids gradually decrease through time as they break down into other compounds. Those which increase through time, i.e. alanine, must be a reaction product, as long as the shell is functioning as a closed system. Alanine is known to be a common product of other amino acid reactions, for instance the decomposition of the relatively unstable amino acids serine, threonine and aspartic acid (Bada and Miller 1970; Bada *et al.* 1978). Alanine is also one of the most stable amino acids (Vallentyne 1964). The production of the proteinogenic amino acid alanine from the decomposition of other amino acids, along with its high stability, explains its anomalously high concentration in these fossils and in others (e.g. Bada and Man 1980).

TAXONOMIC IMPLICATIONS

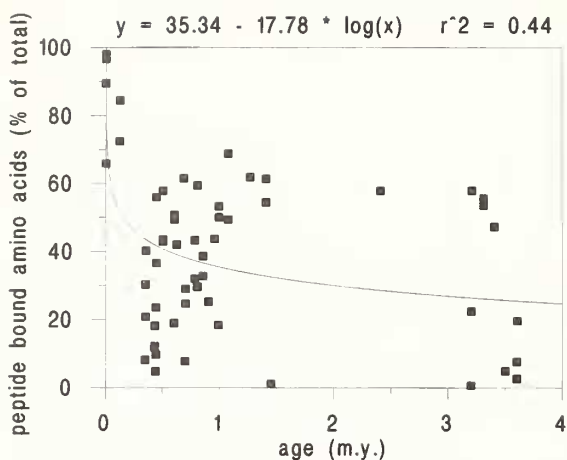
The results of principal component analysis show that samples can be grouped according to their amino acid compositions in ways which reflect their taxonomic groupings (Text-fig. 9). These relationships do not seem to break down due to amino acid diagenesis as the fossils get older, at least within the period examined in this study. This may be because the diagenesis reactions in all samples are very similar, involving the gradual decomposition of most amino acids and the production of diagenetic alanine and various other compounds. Individual standard amino acids break down at different rates experimentally, but in mixtures all the reactions are speeded up and the situation is more complicated because decomposition can occur through many more pathways. Rate depends on temperature, water, the nature of the residues on either side of the bond, and the presence of other compounds (Ikawa and Snell 1954; Vallentyne 1964, 1968; Ho 1966; Kleef *et al.* 1975; Kahne and Still 1988; Eglinton and Logan 1991). Some of the amino acids most stable to pyrolysis have been shown to be some of the least stable when fossilized (Jones and Vallentyne 1960). In this study, most amino acids appear to decompose at fairly similar rates despite their differing thermal stabilities. This means that the proportions of most individual amino acids present within shells of different species are relatively unaffected by diagenesis. The process is ubiquitous in



TEXT-FIG. 6. For caption see opposite.



TEXT-FIG. 6. Amount of free individual intracrystalline amino acids plotted against sample age.



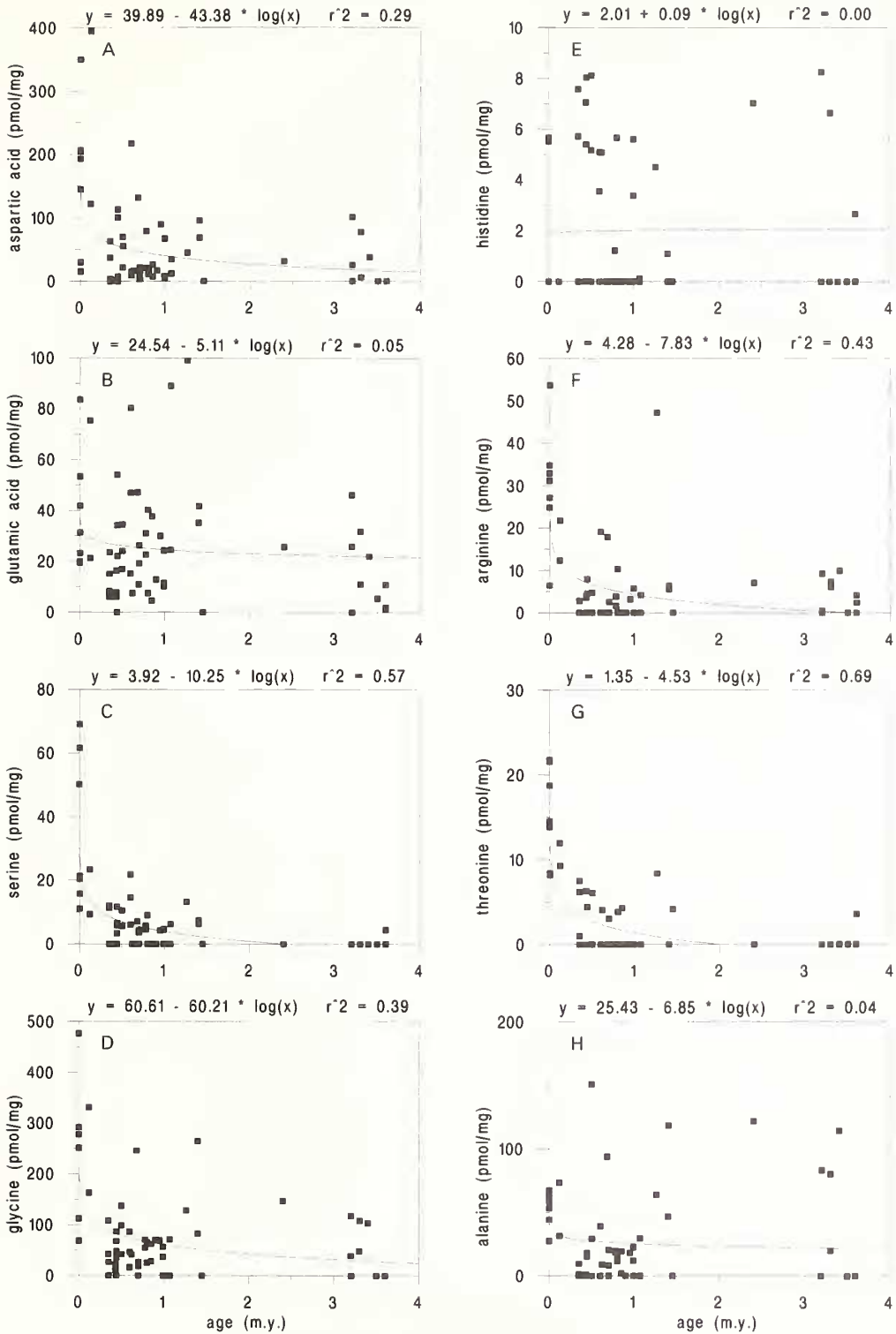
TEXT-FIG. 7. Peptide bound intracrystalline amino acids as a percentage of the total intracrystalline amino acids in all samples through time.

all samples so does not affect relationships, as long as there is enough of the original amino acid left to give a taxonomic signature. When this is finally lost, the taxonomic relationships will quickly break down.

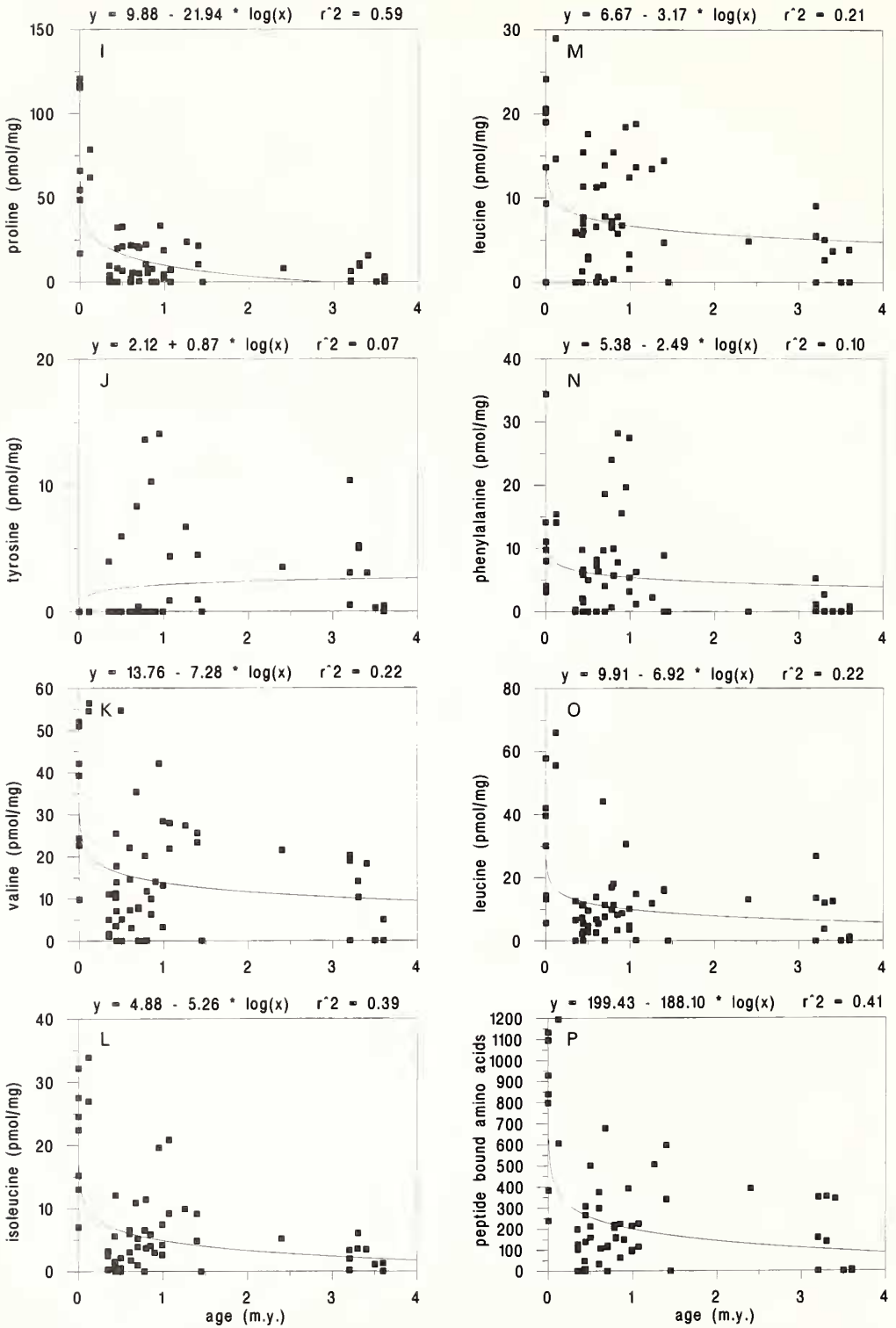
Some morphologically similar groups plot together (Text-fig. 9). For example, samples from the superfamily Ostreacea (*Tiostrea chilensis lutaria* and *Crassostrea ingens*) and Anomiacea (*Patro undatus*) all have an oyster-like appearance. Although the order of amino acids in the shell matrix proteins is derived directly from the DNA and therefore reflects genotype rather than phenotype, the shell morphology is in turn controlled by the shell matrix proteins (Lowenstam and Weiner 1989), so would be expected to follow the same relationships. Therefore, it is interesting to speculate that whilst the overall genotypes of unrelated species may be very different from each other, in cases of similar shell morphology in unrelated species the sections of DNA which control the production of shell matrix proteins may be very similar. This may be a case of reconvergent evolution, resulting in the reconvergence of the morphologies of species that had previously diverged. Therefore, the use of amino acids from shell matrix proteins, although it reflects genotype, may only reflect the part of the genotype which controls shell morphology. The taxonomic relationships inferred from intracrystalline amino acids, therefore, may only reinforce relationships already inferred using the physical measurements of shell morphology. Walton (1992) showed that the intracrystalline amino acid assemblages of recent brachiopods reinforced existing (morphological) taxonomy. However, considering the lack of soft parts available from fossils, and the general lack of preserved DNA, the intracrystalline amino acid compositions of hard parts may nonetheless be one of the best indications of genotype available in the fossil record.

CONCLUSIONS

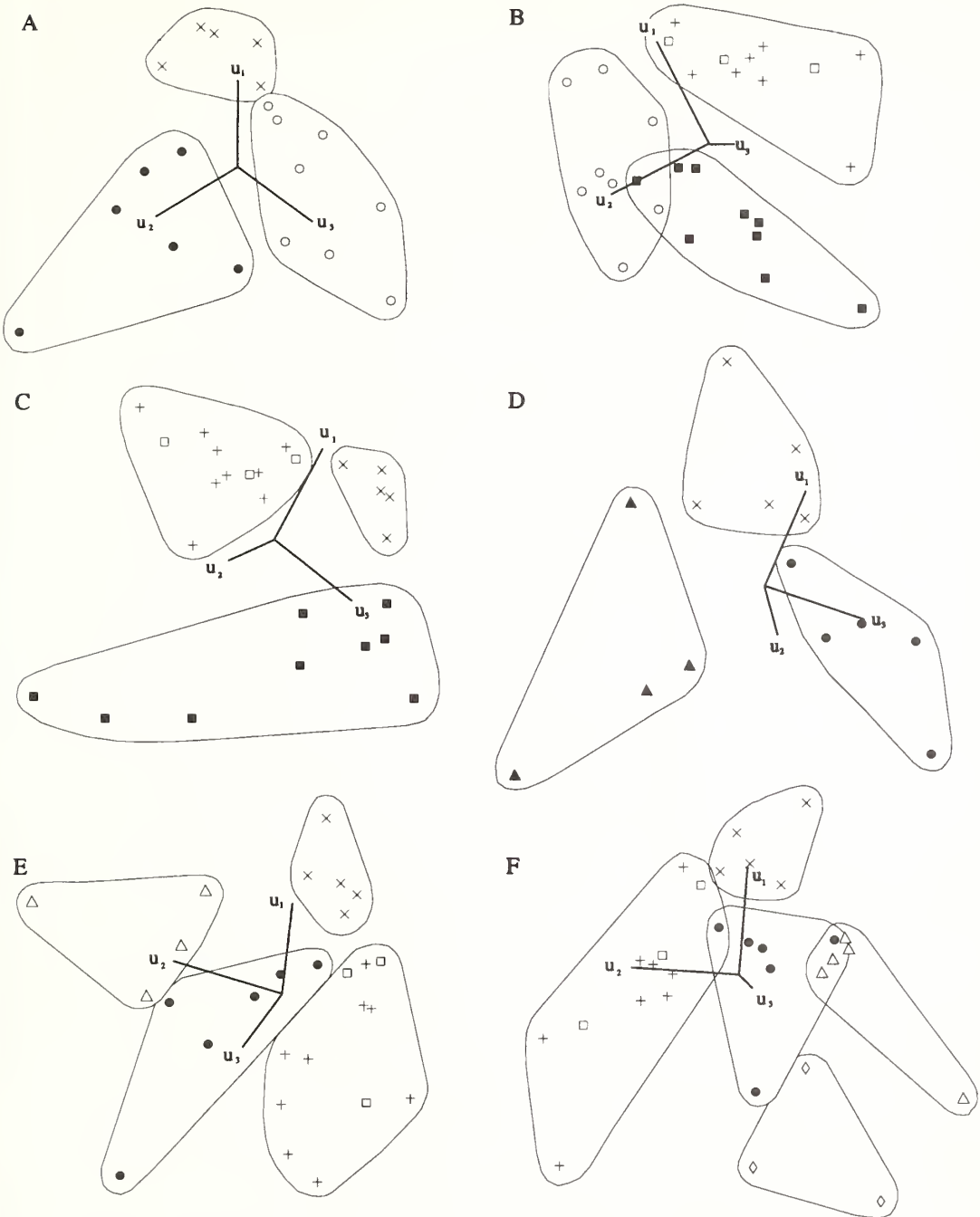
There is a gradual decline in most intracrystalline amino acids through time as they decompose to other compounds. Alanine shows an increase (at least over the time scale of this study) because it is a common product of other amino acid decomposition reactions. In Recent shells, most amino acids are still peptide bound and the number of free amino acids is small. However, most of the peptide bonds are broken within the first 0.5 My. Free amino acids are therefore at a maximum at about 0.5–1 Ma after which there is a decline, except in alanine which continues to increase as other amino acids are broken down. The amino acid diagenesis reactions are fairly ubiquitous in all samples and most intracrystalline amino acids decompose at quite similar rates. Taxonomic relationships can still therefore be inferred from intracrystalline amino acid compositions even after amino acid diagenesis reactions, as long as there is enough original amino acid left to give a taxonomic signal. The results of multivariate analysis of the amino acid compositions of different species tend to reinforce morphological taxonomy.



TEXT-FIG. 8. For caption see p. 868.



TEXT-FIG. 8. Amount of peptide bound individual intracrystalline amino acids plotted against sample age.



□ Anomiacea ○ Carditacea ◇ Mactracea + Ostreacea ▲ Veneracea
 × Cardiacea △ Cerithiacea ■ Mesodesmatacea ● Pectinacea

TEXT-FIG. 9. Three dimensional rotating plots of the first three principal components of amino acid data for various mollusc superfamilies. A, Cardiacea, Carditacea and Pectinacea. B, Carditacea, Mesodesmatacea, Ostreacea and Anomiacea. C, Cardiacea, Mesodesmatacea, Ostreacea and Anomiacea. D, Veneracea, Cardiacea and Pectinacea. E, Pectinacea, Cardiacea, Cerithiacea, Ostreacea and Anomiacea. F, Mactracea, Pectinacea, Cerithiacea, Cardiacea, Ostreacea and Anomiacea.

APPENDIX 1: TOTAL INTRACRYSTALLINE AMINO ACIDS (pmol/mg)

Sample	Age	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Iso	Leu	Phe	Lys	Total
1	3-6	0	5-58	0	30-47	7-66	5-97	0	15-98	9-96	0	4-99	0	3-88	0	0	84-49
2	3-6	36-33	21-01	4-44	78-39	0	11-19	3-56	165-9	15-45	4-35	35-83	9-32	12-58	7-52	12-2	418
3	3-6	42-11	23-26	0	87-23	0	4-13	0	75-39	11-58	5-85	12-33	6-49	6-49	5-53	4-17	284
4	3-5	0	9-6	0	46-23	0	0	0	36-61	14-01	4-19	15-3	5-77	6-14	0	2	140-9
5	0-35	94-43	32-2	12-05	137-1	0	8-42	6-2	95-63	21-75	3-98	22-25	7-94	28-36	3-65	25-52	499-5
6	0	15-52	22-67	15-89	130	0	27-16	8-38	78-36	54-54	0	22-66	13-01	20-61	8	13-23	430-1
7	0	144-6	19-47	21-39	260-3	5-51	31-17	21-57	70-05	116-9	0	51-01	27-52	19-03	11-05	57-84	857-4
8	0-7	94-79	19-18	4-34	166-2	0	11-32	7-88	119-7	34-24	3-06	36-17	7-52	7-53	3-86	20-48	536-3
9	0	193	41-84	61-71	498-3	5-68	34-8	21-75	86-23	115-4	0	42-12	22-41	20-14	14-13	14-43	1172
10	0	349-7	83-72	69-14	308-2	0	53-8	18-71	81-7	66-14	0	39-26	15-21	0	4-09	42-08	1132
11	0	30-44	35-13	16-1	111-1	0	10-43	8-14	54-99	36-79	0	13-6	7-05	26-06	6-05	8-85	364-7
12	3-2	3-19	8-91	0	52-47	0	4-17	0	40-24	17-12	4-78	19-76	9-16	7-24	7-13	2-18	176-4
13	1-45	47-9	13-47	0	99	0	10-02	4-19	126	22-82	3-58	26-13	6-17	4-78	0	8-99	373
14	0	204-3	57-31	50-15	268-1	0	32-94	13-85	50-5	48-83	0	24-35	24-54	9-34	9-75	30-13	824-1
15	0	206-9	26-01	20-46	292-5	0	24-73	14-51	65-39	120-9	0	52-06	32-2	24-16	34-44	39-65	953-8
16	0-12	167	26-07	9-4	221	0	17-9	9-29	73-97	90-77	5-93	71-84	31-42	23-08	17-64	73-04	838-3
17	0-12	410-5	81-58	23-46	386-6	0	28-67	11-91	120-7	116-6	0	77-19	39-99	39-8	18-51	64-27	1420
18	0-35	20-38	15-09	0	60-67	5-71	8-61	5-88	56-91	79-91	0	24-81	9-77	7-16	5-91	15-51	316-3
19	0-35	11-16	10-34	0	404-6	0	0	0	63-93	22-54	0	24-27	4-48	6-09	3-18	3-45	554-1
20	0-35	86-24	28-66	11-37	115-2	7-58	0	7-51	92-47	27-98	0	18-71	6-47	26-41	3-81	19-94	452-3
21	0-43	34-04	27-38	0	157-2	0	10-08	0	171-8	57-49	0	64-07	8-22	49-57	22-08	15-33	617-2
22	0-43	44-09	14-57	0	85-64	6-05	13-78	7-83	83-33	73-12	3-56	61-47	20-76	21-63	10-04	32-08	478
23	0-43	14-48	13-45	0	59-65	0	0	6-32	41-96	26-85	0	16-48	5-96	7-42	13-45	12-05	218-1
24	0-44	87-79	25-69	5-07	253-5	8-04	8-55	5-11	149-7	88-55	8-53	57-74	16-85	20-93	6-64	32-72	775-4
25	0-44	86-26	25-77	10-54	140-8	0	0	0	132-1	16-41	0	27-02	10-9	26-54	5-8	20-99	503-2
26	0-44	140-7	50-48	12-29	334-7	0	0	0	134-4	35-1	4-64	41-45	7-99	12-97	4-41	20-91	799-9
27	0-44	178-9	69-58	11-75	210-7	7-05	11-95	3-94	120-9	87-22	2-62	66-06	25-89	29-23	11-46	40-55	899-8
28	0-44	111-1	44-68	6-61	107-2	10-74	7-94	4-41	63-43	45-13	2-84	41-7	7-84	15-92	3-25	14-59	487-3
29	0-5	135-7	33-56	10-62	359-3	0	0	6-08	177-4	43-67	5-97	54-7	6-93	11-13	3-77	17-39	866-2
30	0-5	71-28	47-12	14-9	102-5	8-12	4-77	0	48-74	35-2	0	17-71	6-82	19-76	4-28	9-88	391-1
31	0-5	39-41	23-51	5-8	154-8	16-36	3-82	6-33	87-64	89	3-59	39-09	15-2	24-21	5-02	26-37	540-1

32	0.6	275.4	93.56	21.87	149.7	0	0	111.2	25.35	4.02	34.53	13.08	37.1	5.44	35.54	806.7
33	0.6	28.98	31.53	6.05	100.6	5.09	9.93	68.41	32.37	3.29	24.17	10.21	26.31	18.32	13.07	378.4
34	0.6	31.58	67.97	14.6	131.8	12.91	44.17	86.67	66.45	6.09	42.4	15.45	22.48	41.76	25.06	609.4
35	0.62	24.32	12.41	0	77.5	10.65	0	37.9	35.39	0	13.12	5.66	5.67	14.34	12.64	253.7
36	0.68	186.9	56.06	11.58	385.0	0	22.95	199.8	45.09	22.85	58.75	17.84	20.42	13.63	70.78	111.2
37	0.7	23.43	37.75	0	107	0	3.89	71.99	44.44	4.2	23.1	10.85	48.14	13.33	15.75	403.9
38	0.7	24.9	33.3	8.31	76.53	0	6.63	75.5	97.82	19.42	49.17	23.18	34.1	63.06	43.44	555.4
39	0.78	114.1	40.41	4.61	188.5	0	8	163	85.5	39.1	74	26.11	26.84	20.42	41.78	832.3
40	0.78	31.34	27.93	5.74	101.6	7.01	3.9	75.11	74.08	5.72	39.19	17.58	20.6	35.21	31.04	476
41	0.85	42.71	44.21	0	163.3	0	0	98.32	52.49	26.78	45.55	16.24	26.13	37.16	23.22	580.4
42	0.85	20.96	8.79	0	69.94	0	0	26.66	31.09	0	14.44	5.91	24.66	11.44	8.97	222.9
43	0.8	23.64	12.23	0	92.57	5.66	0	31.74	23.88	0	15.73	3.7	17.24	9.33	23.73	263.3
44	0.8	20.61	54.74	44.71	98.4	5.2	48.55	125.8	40.94	0	26.11	11.4	43.52	19.28	21.54	560.8
45	0.9	75.73	28.74	0	165.7	0	0	94.51	61.14	0	61.61	18.25	25.67	22.69	34.28	588.3
46	0.95	146	43.94	4.36	190.1	0	7.52	140.4	92.24	38.78	89.1	31.18	37	26.27	55.19	902.1
47	0.99	10.18	15.12	0	59.83	5.6	0	28.49	18.02	0	12.53	4.21	11.49	5.37	5.77	176.6
48	0.99	141.6	35.46	4.44	227.1	0	5.17	135	30.23	3.06	36.22	7.84	7.71	6.13	31.05	671
49	0.99	18.6	17.64	4.67	94.75	11.1	5.72	57.64	73.76	3.1	48.76	17.93	22.43	29.3	24.95	434.3
50	1.07	17.06	101.3	0	7.96	6.35	0	20.26	6.88	4.37	37.59	20.84	31.99	9.13	19.31	283
51	1.07	54.79	31.19	6.3	143.8	0	4.23	111.6	20.93	3.93	21.91	16	23.56	4.16	14.52	456.8
52	1.26	60.59	115.5	13.26	192.7	10.41	58.26	136.4	84.51	9.7	51.03	20.46	22.55	8.02	24.99	816.7
53	1.4	132.6	45.66	6.39	387.5	0	15.39	245.7	36.09	10.04	45.04	11.73	8.68	3.56	25.25	973.6
54	1.4	86.81	39.17	7.48	130.2	7.77	11.85	114.1	76.49	5.27	58.05	21.04	32.66	12.59	30.1	633.6
55	2.4	44.18	29.32	0	216	7.03	15.75	257.5	33.6	7.32	34.14	13.87	4.86	2.68	19.23	685.4
56	3.3	98.32	34.88	0	194.3	0	23.08	203.6	30.3	5.21	32.71	11.18	6.61	3.17	19.13	662.5
57	3.2	43.03	34.87	0	197.3	8.24	9.25	183.7	18.8	10.37	32.3	8.86	15.83	8.93	31.04	604.5
58	3.2	142	53.4	0	291.9	0	22.32	388.3	17.04	9.64	54.54	8.9	11.75	5.79	30.56	1036
59	3.3	5.96	15.47	0	78.57	6.64	6.09	47.25	30.97	5	21.6	9.29	10.69	5.58	6.35	249.5
60	3.4	75.49	28.25	0	215.5	0	21.57	273.3	34.96	3.05	35.06	9.09	7.45	3.15	22.18	729

APPENDIX I: TOTAL INTRACRYSTALLINE AMINO ACIDS (pmol/mg)

Sample	Age	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Iso	Leu	Phe	Lys	Total
1	3-6	0	5-58	0	30-47	7-66	5-97	0	15-98	9-96	0	4-99	0	3-88	0	0	84-49
2	3-6	36-33	21-01	4-44	78-39	0	11-19	3-56	165-9	15-45	4-35	35-83	9-32	12-58	7-52	12-2	418
3	3-6	42-11	23-26	0	87-23	0	4-13	0	75-39	11-58	5-85	12-33	5-94	6-49	5-53	4-17	284
4	3-5	0	9-6	0	46-23	0	0	0	36-61	14-01	4-19	15-3	6-77	6-14	0	2	140-9
5	0:35	94-43	32-2	12-05	137-1	0	8-42	6-2	95-63	21-75	3-98	22-25	7-94	28-36	3-65	25-52	499-5
6	0	15-52	22-67	15-89	130	0	27-16	8-38	78-36	54-54	0	22-66	13-01	20-61	8	13-23	430-1
7	0	144-6	19-47	21-39	260-3	5-51	31-17	21-57	70-05	116-9	0	51-01	27-52	19-03	11-05	57-84	857-4
8	0-7	94-79	19-18	4-34	166-2	0	11-32	7-88	119-7	34-24	3-06	36-17	7-52	7-53	3-86	20-48	536-3
9	0	193	41-84	61-71	498-3	5-68	34-8	21-75	86-23	115-4	0	42-12	22-41	20-14	14-13	14-43	1132
10	0	349-7	83-72	69-14	308-2	0	53-8	18-71	81-7	66-14	0	39-26	15-21	0	4-09	42-08	1172
11	0	30-44	35-13	16-1	111-1	0	10-43	8-14	54-99	36-79	0	13-6	7-05	26-06	6-05	8-85	364-7
12	3-2	3-19	8-91	0	52-47	0	4-17	0	40-24	17-12	4-78	19-76	9-16	7-24	7-13	2-18	176-4
13	1-45	47-9	13-47	0	99	0	10-02	4-19	126	22-82	3-58	26-13	6-17	4-78	0	8-99	373
14	0	204-3	57-31	50-15	268-1	0	32-94	13-85	50-5	48-83	0	24-35	24-54	9-34	9-75	30-13	824-1
15	0	206-9	26-01	20-46	292-5	0	24-73	14-51	65-39	120-9	0	52-06	32-2	24-16	34-44	39-65	953-8
16	0-12	167	26-07	9-4	221	0	17-9	9-29	73-97	90-77	5-93	71-84	31-42	23-08	17-64	73-04	838-3
17	0-12	410-5	81-58	23-46	386-6	0	28-67	11-91	120-7	116-6	0	77-19	39-99	39-8	18-51	64-27	1420
18	0-35	20-38	15-09	0	60-67	5-71	8-61	5-88	56-91	79-91	0	24-81	9-77	7-16	5-91	15-51	316-3
19	0-35	11-16	10-34	0	404-6	0	0	0	63-93	22-54	0	24-27	4-48	6-09	3-18	3-45	554-1
20	0-35	86-24	28-66	11-37	115-2	7-58	0	7-51	92-47	27-98	0	18-71	6-47	26-41	3-81	19-94	452-3
21	0-43	34-04	27-38	0	157-2	0	10-08	0	171-8	57-49	0	64-07	8-22	49-57	22-08	15-33	617-2
22	0-43	44-09	14-57	0	85-64	6-05	13-78	7-83	83-33	73-12	3-56	61-47	20-76	21-63	10-04	32-08	478
23	0-43	14-48	13-45	0	59-65	0	0	6-32	41-96	26-85	0	16-48	5-96	7-42	13-45	12-05	218-1
24	0-44	87-79	25-69	5-07	253-5	8-04	8-55	5-11	149-7	88-55	8-53	57-74	16-85	20-93	6-64	32-72	775-4
25	0-44	86-26	25-77	10-54	140-8	0	0	0	132-1	16-41	0	27-02	10-9	26-54	5-8	20-99	503-2
26	0-44	140-7	50-48	12-29	334-7	0	0	0	134-4	35-1	4-64	41-45	7-99	12-97	4-41	20-91	799-9
27	0-44	178-9	69-58	11-75	210-7	7-05	11-95	3-94	120-9	87-22	24-62	66-06	25-89	29-23	11-46	40-55	899-8
28	0-44	111-1	44-68	6-61	107-2	10-74	7-94	4-41	63-43	45-13	2-84	41-7	7-84	15-92	3-22	14-59	487-3
29	0-5	135-7	33-56	10-62	359-3	0	0	6-08	177-4	43-67	5-97	54-7	6-93	11-13	3-75	17-39	866-2
30	0-5	71-28	47-12	14-9	102-5	8-12	4-77	0	48-74	35-2	0	17-71	6-82	19-76	4-28	9-88	391-1
31	0-5	39-41	23-51	5-8	154-8	16-36	3-82	6-33	87-64	89	3-59	39-09	15-2	24-21	5-02	26-37	540-1
32	0-6	275-4	93-56	21-87	149-7	0	0	0	111-2	25-35	4-02	34-53	13-08	37-1	5-44	35-54	806-7
33	0-6	28-98	31-53	6-05	100-6	5-09	9-93	0	68-41	32-37	3-29	24-17	10-21	26-31	18-32	13-07	370-4
34	0-6	31-58	67-97	14-6	131-8	12-91	44-17	0	86-67	66-45	6-09	42-4	15-45	22-48	41-76	25-06	609-4
35	0-62	24-32	12-41	0	77-5	10-65	0	4-09	37-9	35-39	0	13-12	5-66	5-67	14-34	12-64	253-7
36	0-68	186-9	56-06	11-58	385-0	0	22-95	0	199-8	45-09	22-85	58-75	17-84	20-42	13-63	70-78	1112
37	0-7	23-43	37-75	0	107	0	3-89	0	71-99	44-44	4-2	23-1	10-85	48-14	13-33	15-75	403-9
38	0-7	24-9	33-3	8-31	76-53	0	6-63	0	75-5	97-82	19-42	49-17	23-18	34-1	63-06	43-44	555-4
39	0-78	114-1	40-41	4-61	188-5	0	8	0	163	85-5	39-1	74	26-11	26-84	20-42	41-78	832-3
40	0-78	31-34	27-93	5-74	101-6	7-01	3-9	0	75-11	74-08	5-72	39-19	17-58	20-6	35-21	31-04	476
41	0-85	42-71	44-21	0	163-3	0	0	4-29	98-32	52-49	26-78	45-55	16-24	26-13	37-16	23-22	580-4
42	0-85	20-96	8-79	0	69-94	0	0	0	26-66	31-09	0	14-44	5-91	24-66	11-44	8-97	222-9
43	0-8	23-64	12-23	0	92-57	5-66	0	3-87	31-74	23-88	0	15-73	3-7	17-24	9-33	23-73	263-3
44	0-8	20-61	54-74	44-71	98-4	5-2	48-55	0	125-8	40-94	0	26-11	11-4	43-52	19-28	21-54	560-8
45	0-9	75-73	28-74	0	165-7	0	0	0	94-51	61-14	0	61-61	18-25	25-67	22-69	34-28	588-3
46	0-95	146	43-94	4-36	190-1	0	7-52	0	140-4	92-24	38-78	89-1	31-18	37	26-27	55-19	902-1
47	0-99	10-18	15-12	0	59-83	5-6	0	0	28-49	18-02	0	12-53	4-21	11-49	5-37	5-77	176-6
48	0-99	141-6	35-46	4-44	227-1	0	5-17	0	135	30-23	3-06	36-22	7-84	7-71	6-13	31-05	671
49	0-99	18-6	17-64	4-67	94-75	11-1	5-72	3-91	57-64	73-76	3-1	48-76	17-93	22-43	29-3	24-95	434-3
50	1-07	17-06	101-3	0	7-96	6-35	0	0	20-26	6-88	4-37	37-59	20-84	31-99	9-13	19-31	283
51	1-07	54-79	31-19	6-3	143-8	0	4-23	0	111-6	20-93	3-93	21-91	16	23-56	4-16	14-52	456-8
52	1-26	60-59	115-5	13-26	192-7	10-41	58-26	8-39	136-4	84-51	9-7	51-03	20-46	22-55	8-02	24-99	816-7
53	1-4	132-6	45-66	6-39	387-5	0	15-39	0	245-7	36-09	10-04	45-04	11-73	8-68	3-56	25-25	973-6
54	1-4	86-81	39-17	7-48	130-2	7-77	11-85	0	114-1	76-49	5-27	58-05	21-04	32-66	12-59	30-1	633-6
55	2-4	44-18	29-32	0	216	7-03	15-75	0	257-5	33-6	7-32	34-14	13-87	4-86	2-68	19-23	685-4
56	3-3	98-32	34-88	0	194-3	0	23-08	0	203-6	30-3	5-21	32-71	11-18	6-61	3-17	19-13	662-5
57	3-2	43-03	34-87	0	197-3	8-24	9-25	0	185-7	18-8	10-37	32-3	8-86	15-83	8-93	31-04	604-5
58	3-2	142	53-4	0	291-9	0	22-32	0	388-3	17-04	9-64	54-54	8-9	11-75	5-79	30-56	1036
59	3-3	5-96	15-47	0	78-57	6-64	6-09	0	47-25	30-97	5	21-6	9-29	10-69	5-58	6-35	249-5
60	3-4	75-49	28-25	0	215-5	0	21-57	0	273-3	34-96	3-05	35-06	9-09	7-45	3-15	22-18	729

APPENDIX 2: FREE INTRACRYSTALLINE AMINO ACIDS (pmol/mg)

Sample	Age	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Iso	Leu	Phe	Lys	Total
1	3-6	0	4-4	0	32-81	5	3-57	0	19-76	8-43	0	0	3-7	0	0	0	77-67
2	3-6	59-84	19-04	0	104-5	0	13-26	0	232-6	15-48	4-25	40-11	9-42	16-31	8-47	11-03	534-3
3	3-6	74-3	12-35	0	90-35	0	0	0	96-67	8-52	5-38	13-24	4-65	6-88	4-69	3-22	320-3
4	3-5	0	4-12	0	52-7	0	0	0	47-48	15-96	3-89	16-04	5-65	7-37	3-99	2-46	159-7
5	0-35	31-31	8-69	0	94-96	0	5-62	0	86-02	17-66	0	11-14	4-7	22-54	4-47	12-81	299-9
6	0	0	2-74	0	17-45	0	0	0	24-96	0	0	0	0	0	0	0	45-15
7	0	0	0	0	9-24	0	0	0	7-94	0	0	0	0	0	0	0	17-18
8	0-7	72-9	8-26	0	217-3	0	11-7	4-82	180	33-77	13-03	68-94	6-51	10-54	4-67	20-62	653-1
9	0	0	0	0	21-21	0	0	0	18-88	0	0	0	0	0	0	0	40-09
10	0	0	0	0	16-18	0	0	0	20-5	0	0	0	0	0	0	0	36-68
11	0	0	3-77	4-93	42-13	0	3-97	0	27-66	19-78	0	3-78	0	12-4	2-91	3-14	124-5
12	3-2	4-7	11-13	4-77	100-5	0	3-7	0	74-28	22-81	4-26	26-24	8-99	13-07	7-08	7-05	288-6
13	1-45	156-4	15-05	0	276-3	0	14-4	0	271-1	25-97	8-57	34-68	6-82	9-87	3-87	15-71	838-7
14	0	0	3-91	0	16-15	0	0	0	6-24	0	0	0	0	0	0	0	26-3
15	0	0	2-73	0	14-56	0	0	0	6-63	0	0	0	0	0	0	0	23-92
16	0-12	45-25	4-82	0	57-84	0	5-6	0	42-18	28-69	7	17-21	4-42	8-44	3-55	7	232
17	0-12	16-16	6-08	0	55-66	0	6-95	0	47-18	37-79	6-04	20-77	6-01	10-81	3-13	8-68	225-3
18	0-35	16-91	6-8	0	62-99	0	9-55	4-9	82-8	77-16	10-51	23-08	7-07	7-31	5-6	17-88	332-6
19	0-35	11-3	4-2	0	296-4	0	0	0	65-85	24	8	23-21	4-2	6-5	3-91	3-8	451-4
20	0-35	48-75	13-5	0	88-62	0	5-62	0	91-24	18-23	3-72	13-69	3-99	20-4	4-15	13-31	325-2
21	0-43	32-42	10-97	0	135-8	7-31	6-44	4-97	170-7	69-58	3-38	53-78	6-7	43-92	15-86	8-01	569-8
22	0-43	42-28	6-76	0	74-78	6-83	9-79	10-71	96-06	78-16	32-17	50-22	15-16	20-32	7-92	20-73	471-9
23	0-43	13-77	7-28	0	49-2	0	5-12	0	47-92	57-81	4-9	12-98	5-53	7-69	3-68	9-86	225-7
24	0-44	80-38	26-4	8-29	166-6	0	15-56	7-15	131-8	56-22	30-52	43-76	26-3	13-99	0	29-86	636-9
25	0-44	97-67	31-09	7-17	166-2	0	4-9	0	197	36-27	8-32	31-11	43-04	11-09	0	23-79	657-7
26	0-44	141-7	28-44	6-38	299-7	0	3-97	0	175-5	38-2	10-88	34-34	18-08	6-86	2-92	22-86	789-8
27	0-44	65-66	15-66	0	142-7	0	7-41	4-2	125-6	67-25	39-6	48-25	13-81	21-5	9-65	29-02	590-3
28	0-44	10-61	10-52	0	59-2	5-34	0	0	48-26	36-9	5-36	16-22	10-58	4-55	4-28	9-14	221
29	0-5	65-33	9-55	0	221-7	0	0	0	26-4	10-87	0	0	6-43	8-03	4-1	12-74	365-2
30	0-5	15-76	12-65	9-19	60-51	0	0	0	50-52	28-42	0	12-64	4-71	17-01	13-3	7-16	231-9
31	0-5	17-84	6-48	0	55-52	11-2	5-42	7-97	58-41	67-96	12-71	42-9	17-47	6-61	0	16-71	327-2